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I hereby certify that this **TRANSMITTAL LETTER, CONTINUATION PATENT APPLICATION, PRELIMINARY AMENDMENT AND FILING FEE** are being deposited with the United States Postal Service by Express Mail Procedure in an envelope addressed to: Assistant Commissioner for Patents, Box Patent Application, Washington, D.C. 20231.

Chuck Dunbar

Form 3.54 Division-continuation
program application transmittal form
37 C.F.R. 1.60

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Docket No. BKY 2 040-1-1-1

Prior application: 08/624,374
Examiner: Geetha P. Bansal
Art Unit: 1642

The Assistant Commissioner for Patents
Washington, D.C. 20231
Box Patent Application

Sir:

This is a request for filing a continuation application under 37 C.F.R. 1.60, of pending prior application **U.S. Serial No. 08/624,374 filed on March 27, 1996** of Gavin Paul Vinson et al.

For: **TYPE I ANGIOTENSION II RECEPTOR SPECIFIC MONOCLONAL ANTIBODIES AND HYBRIDOMAS**

1. xxx Enclosed is a complete copy of the prior application, including the oath or declaration as originally filed and a verified statement claiming small entity status - nonprofit organization.

2. xxx The filing fee is calculated below.

CLAIMS AS FILED IN THE PRIOR APPLICATION LESS ANY CLAIMS CANCELLED BY PRELIMINARY AMENDMENT

CLAIMS AS FILED IN THE PRIOR APPLICATION LESS ANY CLAIMS CANCELED BELOW			
Basic Filing Fee (Small Entity)			$345.00
		No. of Extra Claims Present	Additional Rate
Total Claims	13 - 20	0	$ 0.00
Indep. Claims	4 - 3	1	$ 39.00

Total fee **$ 384.00**

3. xxx A check in the amount of $ 384.00 is enclosed. Small entity staus was established in the prior application and is still proper.

4. xxx The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Account No. 06-0308. A duplicate copy of this sheet is enclosed.

5. xxx A Preliminary Amendment is enclosed.

6. xxx Priority of British Application Serial No. 9319877.8, filed on September 27, 1993 is claimed under 35 U.S.C. §119. The priority document is contained in the National stage application No. PCT/GB94/02100.

7. xxx The power of attorney in the prior application is to Thomas E. Kocovsky, Reg. No. 28,383; Richard M. Klein, Reg. No. 33,000; and, Jay F. Moldovanyi, Reg. No. 29,678. A copy of the power in the prior application is enclosed. Please address all future communications to

Jay F. Moldovanyi
FAY, SHARPE, FAGAN,
MINNICH & McKEE, LLP
1100 Superior Avenue, 7th Floor
Cleveland, Ohio 44114-2518
(216) 861-5582

I hereby verify that the attached papers are a true copy of prior application **U.S. Serial No. 08/624,374 as originally filed on March 27, 1996**.

The undersigned declare further that all statements made herein of his or her own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

31 Mar. 2000
Date

Jay F. Moldovanyi
Reg. No. 29,678

Attorney of Record

Address of Signator:

FAY, SHARPE, FAGAN,
MINNICH & McKEE, LLP
1100 Superior Avenue, 7th Floor
Cleveland, Ohio 44114
(216) 861-5582

Applicant or Patentee: _____ Attorney's Docket No.: _____
Serial or Patent No.: _____
Filed or Issued: _____
For: _____

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS (37 CFR 1.9 (f) and 1.27 (d)) — NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION Queen Mary & Westfield College
ADDRESS OF ORGANIZATION Mile End Road, London E1 4NS, United Kingdom.

TYPE OF ORGANIZATION

[x] University or other institution of higher education
[ ] Tax exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c) (3))
[ ] Nonprofit scientific or educational under statute of state of The United States of America
(Name of state _____)
(Citation of statute _____)
[ ] Would qualify as tax exempt under Internal Revenue Service Code (26 USC 501(a) and 501(c) (3)) if located in The United States of America
[ ] Would qualify as nonprofit scientific or educational under statute of state of The United States of America if located in The United States of America
(Name of state _____)
(Citation of statute _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 37 CFR 1.9 (e) for purposes of paying reduced fees under section 41(a) and (b) of Title 35, United States Code with regard to the invention entitled NOVEL HYBRIDOMA & MONOCLONAL ANTIBODIES PRODUCED THEREBY by inventor(s) Gavin Paul VINSON and Stewart BARKER described in

[ ] the specification filed herewith
[x] application serial no. PCT/GB94/02100, filed 27TH SEPTEMBER 1994.
[ ] patent no. _____, issued _____.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor, who could not qualify as a small business concern under 37 CFR 1.9 (d) or by any concern which would not qualify as a small business concern under 37 CFR 1.9 (d) or a nonprofit organization under 37 CFR 1.9 (e).

*NOTE: Separate verified statements are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

NAME _____
ADDRESS _____
[ ] INDIVIDUAL [ ] SMALL BUSINESS CONCERN [ ] NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28 (b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME OF PERSON SIGNING M. G. LEONARD
TITLE IN ORGANIZATION External Grants Accountant.
ADDRESS OF PERSON SIGNING Queen Mary & Westfield College, Mile End Road, London E1 4NS, United Kingdom.

SIGNATURE M. G. Leonard DATE 5 3 96

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I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231

Chuck Dunbar

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Gavin Paul Vinson et al.
For:	TYPE I ANGIOTENSION II RECEPTOR SPECIFIC MONOCLONAL ANTIBODIES AND HYBRIDOMAS
Serial No.:	Unknown
Filed:	Herewith
Attorney Docket No.:	BKY 2 040-1-1-1
	Cleveland, Ohio 44114 March 31, 2000

Assistant Commissioner For Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Dear Sir:

Prior to examination of the above-identified U.S. patent application please amend the application as follows:

IN THE SPECIFICATION:

On page 1, before line 1, please add the following new paragraph:

--This application is a continuation of application Serial No. 08/624,374 which was filed on March 27, 1996 and still pending. That application, in turn, is the entry into the National Phase of International Application No. PCT/GB94/02100 which was filed on September 27, 1994.--.

IN THE CLAIMS:

Please amend claims 8-13 as follows:

8. (Amended) Use of the monoclonal antibody according to [any one of the claims 5 to 7] claim 5 for the detection of AT_1 subtype of angiotension II receptor.

9. (Amended) Use of the monoclonal antibody according to [any one of the claims 5 to 7] claim 5 for the control of vaso-constriction.

10. (Amended) Use of the monoclonal antibody according to [any one of the claims 5 to 7] claim 5 for the control of uterine contractions.

11. (Amended) A diagnostic test kit comprising the monoclonal antibody of according to [any one of claims 5 to 7] claim 5 attached to a detectable label.

12. (Amended) A method of treating hypertension comprising administering a therapeutic effective amount of a monoclonal antibody according to [any one of claims 5 to 7] claim 5.

13. (Amended) A method of controlling uterine contractions comprising administering a therapeutic effective amount of a monoclonal antibody according to [any one of claims 5 to 7] claim 5.

Remarks

This application is a continuation of U.S. Application Serial No. 08/624,374 which was filed on March 27, 1996. In this application, applicant is resubmitting the claims as they originally stood at the entry into the National Phase of the International Application. In order to avoid any difficulties

concerning the use of multiple dependent claims, applicant has submitted the instant Preliminary Amendment to remove multiple dependencies from the claims.

Prompt and favorable examination of this application is respectfully requested.

Respectfully submitted,

FAY, SHARPE, FAGAN,
MINNICH & McKEE, LLP

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09534085 . 032100

TYPE I ANGIOTENSIN II RECEPTOR SPECIFIC MONOCLONAL ANTIBODIES AND HYBRIDOMAS.

The present invention relates to a novel hybridoma cell line, in particular it relates to a novel hybridoma cell line which secretes monoclonal antibodies capable of binding to the AT_1 subtype of the Angiotensin II receptor (AT_1 receptor). The invention also relates to monoclonal antibodies secreted by the hybridoma, which antibodies may be used in a test kit having several diagnostic and monitoring applications. It also relates to the use of the monoclonal antibodies in therapeutic applications such as the control of smooth muscle stimulation.

The hormone angiotensin II (Ang II) forms part of the renin - angiotensin system which helps to control electrolyte balance and blood pressure within the body. There are several tissues within the body upon which Ang II acts, they include the adrenal gland, uterus, liver, brain and kidney.

Amongst the several established functions of angiotensin II, it is known to stimulate smooth (unstriated) muscle cell contraction. It stimulates the contraction of smooth muscle cells in the blood vessel wall thus causing vaso-constriction, which leads to hypertension. Most treatments for high blood pressure will include blockage of angiotensin function in one way or another. Smooth muscle also occurs in other locations, for example in the uterus and in the gastrointestinal tract, and elsewhere.

Ang II also stimulates the secretion of aldosterone by the adrenal cortex. Aldosterone is a potent hormone which acts primarily on the kidney to promote sodium retention and thus inter alia, heightens the hypertensive effects of angiotensin acting directly on the vasculature.

Ang II is known to act on various sites in the brain, and one of its actions in animals is the regulation

of thirst and drinking.

Angiotensin also has trophic effects on the vasculature, promoting growth of the muscles in the arterial wall. It is also thought to be angiogenic, i.e. it causes vascularisation of newly developing tissue.

The actions of angiotensin II in cells are mediated through two important intracellular signalling mechanisms. When the hormone binds to its receptor, it activates a specific enzyme, phospholipase C, which acts upon a constituent of the cell membrane called phosphatidyl inositol. This is split by the enzyme into two moieties, called inositol trisphosphate (IP3), and diacylglycerol (DAG). Both of these are involved in eliciting further effects within the target cell. IP3 stimulates increased cellular cytosolic calcium concentrations, which in turn evokes other cellular responses, whereas DAG stimulates another specific enzyme called protein kinase C (PKC).

Most of the established effects of Ang II have been found to occur via the AT_1 subtype of the Ang II receptor, which is a seven transmembrane domain receptor. This receptor has been cloned and sequenced from a variety of tissues, and has been found to be a 359 amino acid polypeptide with a predicted molecular weight of around 40kD (Bernstein and Alexander, (1992), Endocr. Rev., 13, 381-386). Studies using photo-affinity labelling and crosslinking agents have suggested molecular weights for mature receptor of approximately 65kD and 116 kD, respectively, which may reflect glycosylation of asparagine residues within the extra-cellular domain.

Whilst polyclonal antibodies and anti-idiotypic antibodies have been prepared to the Ang II receptor, from which it has been postulated that the receptor has a molecular weight of from 60 to 95 kD, and 63 kD respectively, to date no-one has succeeded in preparing a

monoclonal antibody to this receptor.

It has now been found that by immunising mice with a synthetic peptide corresponding to amino acid residues 8-17 of the rat vascular smooth muscle AT_1 receptor (Murphy, T.J. et al, (1992), Nature, 351, 233-236), and thereafter fusing spleen cells from the immunised mice with mouse myeloma cells, a novel hybridoma cell line is produced, which secretes monoclonal antibodies to the AT_1 subtype of the Ang II receptor.

According to one aspect of the invention there is provided a hybridoma cell line which produces monoclonal antibodies capable of binding to the AT_1 subtype of the angiotensin II receptor. A hybridoma cell line that secretes such monoclonal antibodies was deposited on 22 July 1993 with the European Collection of Animal Cell Cultures, Porton Down, United Kingdom, under the Budapest Treaty, and designated accession No. 93072117.

Such a hybridoma cell line produces antibodies which bind specifically to amino acid residues 8 to 17 of the rat vascular smooth muscle AT_1 receptor. It was found, however, that the monoclonal antibodies would bind to the AT_1 receptor in bovine and human tissue, as well as in rat tissue. To date this sequence of amino acid residues has been found, and is therefore conserved, in all mammalian AT_1 receptors so far cloned. Thus the hybridoma cell line produces antibodies that bind specifically to a peptide having the following amino acid sequence:-

H_2N-Glu- Asp- Gly- Ileu- Lys- Arg- Ileu- Gln- Asp- Asp- -COOH

(Sequence ID No. 1)

According to a second aspect of the invention there is provided a monoclonal antibody that binds to the AT_1 subtype of the angiotensin II receptor. Such monoclonal antibodies bind specifically to amino acid residues 8-17 of

the mammalian AT_1 receptor, that is they bind specifically to a peptide having the amino acid sequence given above.

A hybridoma cell line, according to the invention, can be prepared by immunising inbred mice by techniques well known in the art (Kohler and Milstein, (1975), Nature, 256, 495-497). A peptide was synthesised which corresponds to amino acid residues 8-17 (extracellular) of the published rat vascular smooth muscle AT_1 receptor. The peptide was then conjugated to bovine serum albumin (BSA) and used to immunise mice.

Following a booster injection of the peptide-BSA conjugate the mouse spleens were removed, and the spleenocytes were combined with mouse myeloma cells. Mixed myeloma - lymphocyte hybrids were selected by growth in hypoxanthine, thymidine and aminopterin in an appropriate cell culture medium.

The presence of hybridoma cell lines producing monoclonal antibodies to the AT_1 subtype of the angiotensin II receptor was first detected by screening the hybridoma conditioned medium for binding to a rat liver cell suspension. Such positive binding was detected using peroxidase - conjugated rabbit anti-mouse immunoglobulin (IgG) antibody. Following the initial screening, cell cultures showing positive results were expanded and tested for specific binding to rat adrenal glomerulosa on both frozen sections and dispersed cell smears, using a fluorescein-conjugated rabbit anti-mouse (IgG) antibody. The integrity of the anti-AT_1 receptor monoclonal antibodies produced by the hybridoma was confirmed by binding to rat AT_{1A} receptor transiently expressed by transfected Cos-7 cells.

The monoclonal antibodies of the present invention may be tagged with compounds that fluoresce at various wavelengths, so that the location and distribution of AT_1

receptors in body tissues can be determined by immunohistological techniques. For example, using this monoclonal antibody AT_1 receptors have been found in breast tumours, thus the antibody may be useful in the cancer diagnostic field.

In addition, using the monoclonal antibody of the present invention a hitherto unknown site of action for Ang II has been discovered. It has been found that both rat and human sperm tails express the angiotensin II receptor, and the physiology of the regulation of its expression leads to the possibility that the hormone may be profoundly important in the control of sperm motility, with a potential effect on male fertility. The monoclonal antibodies of the present invention may thus be used in a standard radioimmuno-assay or enzyme-linked immunosorbant assay to study, and possibly measure, sperm motility, as well having a use in contraception.

According to a further aspect of the invention there is provided a diagnostic test kit comprising the monoclonal antibodies of the present invention attached to a detectable label. Such detectable labels include radioisotopes, enzymes and fluorescent compounds.

It has been found that addition of the monoclonal antibodies according to the present invention to living cells inhibits the angiotensin II-generated IP3 response, but it has no action on PKC activation. Thus the antibody interacts highly specifically with just one of the two major signalling pathways stimulated by angiotensin II. This property can be used experimentally to discriminate between the effects of these two pathways, as well as in therapeutic applications. For example, the monoclonal antibodies may be used in controlling vaso-constriction and therefore used to treat hypertension, as well as for the regulation of menstruation and the control of uterine contractions to

prevent miscarriage.

Furthermore these monoclonal antibodies can be used in both immunohistochemistry and immunoelectron-miscroscopy, and may have applications in immunoblotting and immunocytological staining.

The invention is illustrated by the following example.

Example 1

Peptide Synthesis

A peptide was synthesised corresponding to amino acid residues 8-17 (extracellular) of the published rat vascular smooth muscle AT_1 receptor, with the addition of a cysteine residue to facilitate subsequent conjugation to bovine serum albumin (BSA) at either the carboxy-terminal end or the amino terminal end respectively, via an N-Succinimidyl 3-(2-pyridyldithio) proprionate (SPDP) bridge. The synthesis was carried out using FMOC chemistry on an automated synthesiser, followed by reverse phase HPLC. FMOC chemistry is the use of fluorenylmethoxy carbonyl protected amino acids in peptide synthesis.

Immunogen preparation and Immunisation

To prepare the peptide-BSA conjugates, a 0.22mM solution of BSA, in degassed 100mM sodium phosphate buffer containing 100mM sodium chloride (SPSC buffer; pH 7.5), was 'activated' by incubation for 60 min at room temperature with 20mM SPDP (in absolute ethanol) at a ratio of 9:1 (v/v) followed by dialysis in SPSC buffer. 3mg of peptide in 1.2ml SPSC buffer was then incubated with 6ml of 'activated' BSA for 60 min at room temperature, followed by further dialysis in SPSC buffer. Balb C/c mice (8 weeks old) were then immunised by subcutaneous injection with 0.2 ml of a 1:1 emulsion of peptide-BSA conjugate in SPSC buffer (representing approximately 400 μg/ml peptide) and Freund's complete adjuvant. This was followed by a booster injection

four weeks later using a similar emulsion of peptide conjugate in Freund's Incomplete adjuvant. Four days later the mouse spleens were removed.

Production of Hybridomas

Sp2/0-Ag-14 mouse myeloma cells were cultured in RPMI 1640 medium supplemented with 20% (v/v) fetal bovine serum (FBS). These myeloma cells were combined with spleen cells obtained from the immunised mice, and the formation of fused hybrids was aided using 40% (v/v) polyethylene glycol. Mixed myeloma-lymphocyte hybrids were selected by culture in 96- well plates using RPMI containing 20% FBS and hypoxanthine (13.6 mg/1), aminopterin (0.19 mg/1) and thymidine (3.88mg/1) (HAT) (Galfre and Milstein, (1981), Methods Enzymol., 73, 3-46).

After 14 days of HAT selection, hybridoma conditioned medium was removed and screened for binding to a rat liver cell suspension, which had been washed thoroughly with serum-free medium and fixed, using 3.7% (v/v) formaldehyde in 10mM phosphate buffered saline (PBS; pH 7.3), on to poly-L-lysine coated 96-well plates. A peroxidase-conjugated rabbit anti-mouse immunoglobin (IgG) antibody was used to detect positive binding, and this was visualised, as a brown colouration, with diaminobenzidine (DAB) reagent containing imidazole. After initial screening, cells from positive wells were expanded into 24-well plates and cultured in RPMI 1640 containing 20% Myoclone (obtained from Gibco BRL, Uxbridge, UK), and hypoxanthine (13.6mg/1) and thymidine (0.19mg/1), supplemented with 10% v/v thymocyte conditioned medium. Conditioned media from expanded cultures were then tested for specific binding to rat adrenal glomerulosa on both frozen sections and dispersed cell smears, using a fluorescein-conjugated rabbit anti-mouse IgG as described by Laird, S.M, et al in Acta Endocrinologica (Copenh.) (1988)

19, 420-426. Glomerulosa specific populations were then cloned by limiting dilution as described by Goding, J.W. in J. Immunol. Methods, (1980), 39, 285-306.

Transfected AT_1 receptor preparation

Rat AT_{1A} receptors were transiently expressed in COS-7 cells as described by Barker, S., et al in Biochem Biophys. Res. Commun., (1993), 192, 392-398. Cells were sonicated, on ice, in 50mM Tris-HC buffer (pH 7.4) containing aprotinin (1μg/ml), soybean trypsin inhibitor (1μg/ml), phenyl methyl sulfonyl fluoride (30μg/ml and ethylene diamine tetra-acetic acid EDTA (300μg/ml), and centrifuged at 800g for 5 min at 4°C. The resultant supernatant was centrifuged at 100 000g for 1 hour at 4°C. The particulate fraction was then resuspended in the above buffer and diluted to give 100 μg protein/25μl. The Screening and Cloning preparation was then incubated for 30 min at 4°C in the presence of 1% (v/v) Triton x100 (Trade Mark) to solubilise membrane proteins. Sham-transfected COS-7 cells were treated similarly.

Gel electrophoresis and immunoblotting

100μg of solubilised proteins, obtained from the membrane fraction of COS-7 cells, prepared as described above, was loaded in each well and proteins were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) on an 8% gel, running at 200v for approximately 4 hours. Molecular weight markers (106 kD-18.5 kD) were also loaded. Proteins were then electro transferred to Hybond-Enhanced chemiluminescence (ECL) nitrocellulose membranes overnight at 200mA. The membranes were blocked using PBS containaing 10% (w/v) milk protein for 1 hour at room temperature. After washing thoroughly with PBS containing 0.1% (v/v) Tween 20 (Trade Mark) (PBS-T), membranes were incubated with primary antibody (1:20 v/v

in PBS-T) for 1 hour at room temperature. Membranes were washed with PBS-T as before, and then incubated for a further hour at room temperature with horseradish peroxidase-linked sheep anti-mouse Ig antibody, at a dilution of 1:5000 in PBS-T. Following further thorough washing with PBS-T, membranes were treated with ECL reagent and chemiluminescence was detected by exposure to Hyperfilm-ECL.

Results

Amongst the hybridoma clones of interest was one which secreted antibody, and which has been deposited as cell line accession No. 930720117 with the European Collection of Animal Cell Cultrues, Porton Down, UK. To confirm that this cell line truly produced antibody that recognised AT_1 receptor, SDS-PAGE and immunoblotting of solubilised proteins obtained from the membrane fraction of COS-7 cells transfected with rat adrenal AT_{1A} receptor cDNA were carried out as described above. Figure 1 shows that the antibody produced by this cell line identified two prominent protein species with approximate molecular weights of 40 and 60 kG. These were not detected in parallel samples from sham-transfected COS-7 cell controls.

Example 2

Use of monoclonal antibodies to control sperm motility

Human sperm samples were obtained from 12 volunteers and patients attending the Newham Hospital assisted fertility clinic. Samples were suspended in modified minimum essential medium with Earle's salts (MEM) and glutamine and viewed in a Makler chamber using an Olympus inverted microscope fitted with an Olympus ARTF-2 video camera. Fields were recorded on video tape and percentage motility evaluated.

Percentage motility was estimated on playback of the video tapes by freezing the frame to count all of the sperm within a field and then, in forward mode, by counting immotile sperm, i.e. those which within the period of observation did not move to an adjacent square (100 μmetre) on the Makler Chamber grid. In practice, rigid use of this definition was rarely necessary as sperm were either completely immotile or progressed freely.

One series of samples was kept as controls. To a second series Angiotensin II amide (10 nmole/1) was added. A third series was treated with monoclonal antibody to the AT_1 receptor before angiotensin was added.

Velocity was measured by timing forwardly progressive sperm traversing the grid on the Makler Chamber and timing them manually.

From Figure 2(a) it can be seen that stimulation with angiotensin II significantly stimulated forward progressive velocity compared with the untreated controls, whilst addition of the monoclonal antibody inhibited the response to angiotensin II.

From Figure 2(b) it can be seen that similar results were obtained for the effect on the percentage of motile sperm.

Example 3

Effect of monoclonal antibody on smooth muscle contractility in the rat uterus

The uterus was excised from a Wistar rat (350g), and placed in minimum essential medium (MEM) and kept at room temperature. The organ was cleared of fat and a piece of tissue 3cm long was cut from one horn and tied, using sutures, on to a basal isotonic transducer, which was linked to an analogue to digital converter. The resultant signal was analysed by computer using Chart software. A small weight (Approx. 1g) was applied to take up tissue slack. The tissue was then totally immersed in incubation medium (MEM) in an isolated organ bath maintained at 37°C, and incubated until regular contractions of constant amplitude were observed. Fresh medium was applied and the tissue was once again allowed to equilibrate. The effects of angiotensin II, and monoclonal antibody on the rate and amplitude of contraction was assessed by adding these agents directly to the organ bath. The tissue was washed in several changes of medium between different test conditions.

From Figure 3(a) it can be seen that, when angiotensin II at 0.1nmole/l was added, at arrow (A), the amplitude and frequency of subsequent uterine contractions was significantly increased.

From Figure 3(b) it can be seen that subsequent addition of monoclonal antibody, at arrow (B), inhibited the rate and intensity of the contractions to below, not only the angiotensin stimulated level, but also the basal level.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT

(A)	NAME:	Queen Mary & Westfield College
(B)	STREET:	Mile End Road
(C)	CITY:	London
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(A)	NAME:	VINSON, Gavin Paul-Queen Mary & Westfield College
(B)	STREET:	Mile End Road
(C)	CITY:	London
(E)	COUNTRY:	United Kingdom
(F)	POSTAL CODE (ZIP):	E1 4NS

(A)	NAME:	BARKER, Stewart- Queen Mary & Westfield College
(B)	STREET:	Mile End Road
(C)	CITY:	London
(E)	COUNTRY:	United Kingdom
(F)	POSTAL CODE (ZIP):	E1 4NS

(ii) TITLE OF INVENTION: NOVEL HYBRIDOMA & MONOCLONAL ANTIBODIES PRODUCED THEREBY

(iii) NUMBER OF SEQUENCES: 1

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC Compatible
(C) OPERATING SYSTEM: PC - DOS/MS - DOS
(D) SOFTWARE: PatentIn Release # 1.0, Version # 1.25 (EPC

(v) CURRENT APPLICATION DATA:
APPLICATION NUMBER: PCT/GB94/

(vi) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: GB 9319877.8
(B) FILING DATE: 27-SEPT-1993

(2) INFORMATION FOR SEQ ID NO:1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH:
(B) TYPE: amino acid
(C) STRANDEDNESS: unknown
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(vi) ORIGINAL SOURCE:
(H) CELLINE: Hybridoma

(xi) SEQUENCE DESCRIPTION: SEQ ID NO 1:

Glu Asp Gly Ileu Lys Arg Ileu Gln Asp Asp
1 5 10

CLAIMS

1. A hybridoma cell line which produces monoclonal antibodies capable of binding to the AT_1 subtype of the angiotensin II receptor.

2. A hybridoma cell line according to claim 1 which produces monoclonal antibodies that bind specifically to amino acid residues 8-17 of the mammalian AT_1 receptor.

3. A hybridoma cell line which produces monoclonal antibodies that bind specifically to a peptide having the amino acid sequence

H_2N-Glu- Asp- Gly- Ileu- Lys- Arg- Ileu- Gln- Asp- Asp- -COOH

4. A hybridoma cell line according to claim 1 being characterised by cell line accession No. 930720117 deposited at European Collection of Animal Cell Cultures, Porton Down, UK.

5. A monoclonal antibody that binds to the AT_1 subtype of the angiotensin II receptor.

6. A monoclonal antibody according to claim 5 that binds to amino acid residues 8-17 of the mammalian AT_1 receptor.

7. A monoclonal antibody that binds specifically to a peptide having the amino acid sequence

H_2N-Glu- Asp- Gly- Ileu- Lys- Arg- Ileu- Gln- Asp- Asp- -COOH

8. Use of the monoclonal antibody according to any

one of claims 5 to 7 for the detection of AT_1 subtype of angiotensin II receptor.

9. Use of the monoclonal antibody according to any one of claims 5 to 7 for the control of vaso-constriction.

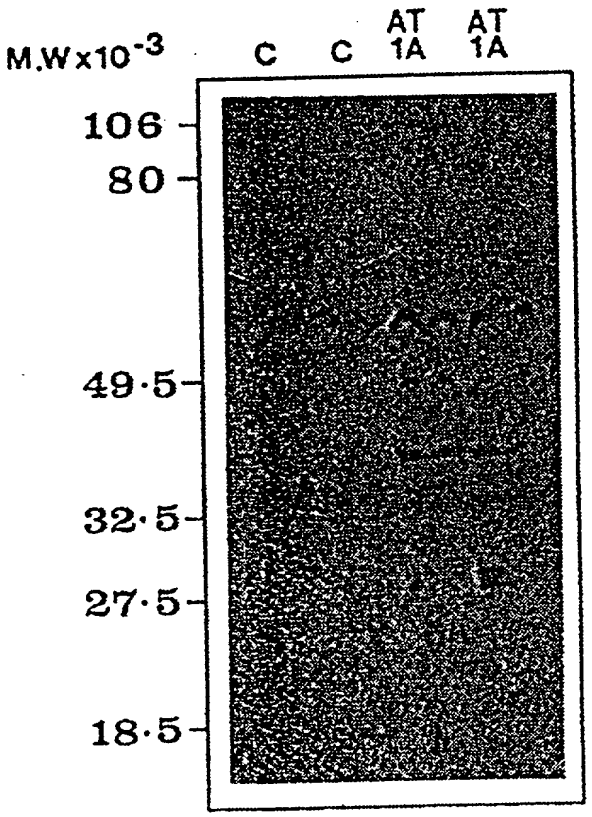
10. Use of the monoclonal antibody according to any one of claims 5 to 7 for the control of uterine contractions.

11. A diagnostic test kit comprising the monoclonal antibody of according to any one of claims 5 to 7 attached to a detectable label.

12. A method of treating hypertension comprising administering a therapeutic effective amount of a monoclonal antibody according to any one of claims 5 to 7.

13. A method of controlling uterine contractions comprising administering a therapeutic effective amount of a monoclonal antibody according to any one of claims 5 to 7.

FIG. 1



Immunoblot of solubilised membrane proteins from control COS- 7 cells (C), and COS- 7 cells expressing rat adrenal AT_{1A} receptors using anti- AT_1 monoclonal antibody produced by hybridoma cell line No. 930720117

FIG. 2(a)

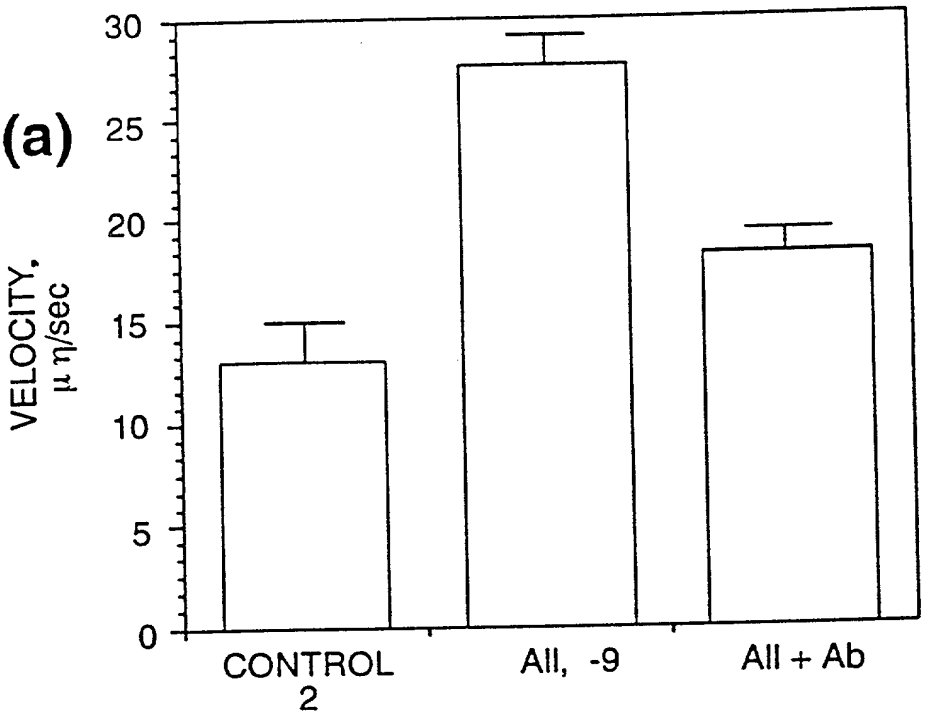
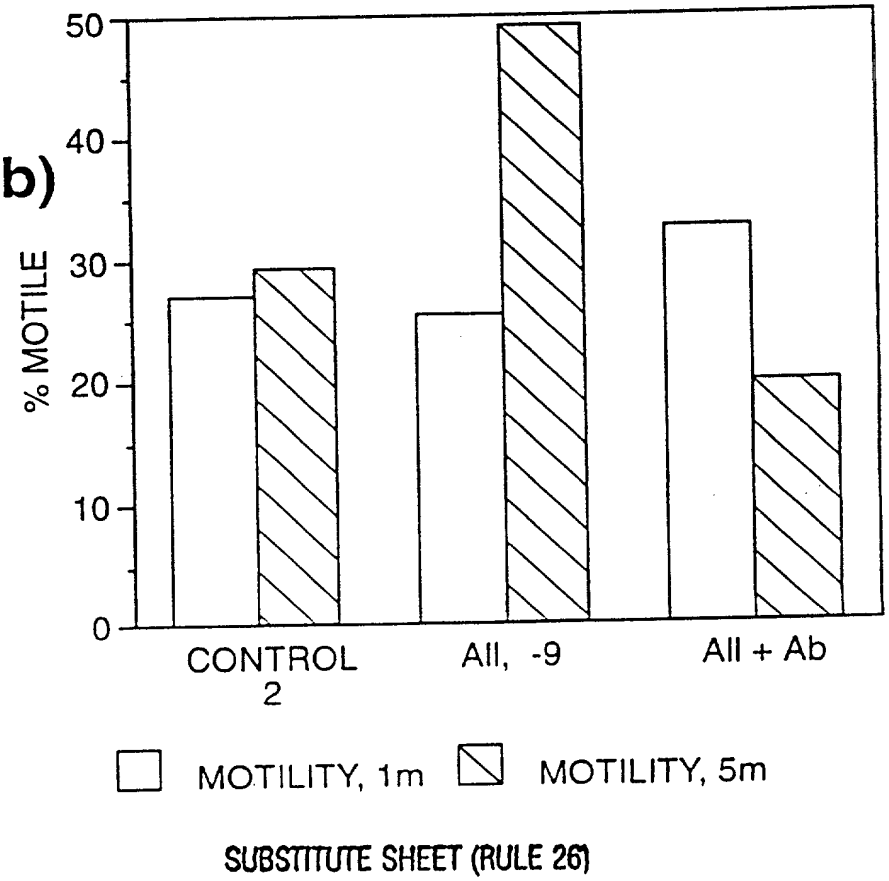

VELOCITY, μ η/sec
30
25
20
15
10
5
0
CONTROL 2
All, -9
All + Ab

FIG. 2(b)


% MOTILE
50
40
30
20
10
0
CONTROL 2
All, -9
All + Ab
MOTILITY, 1m
MOTILITY, 5m

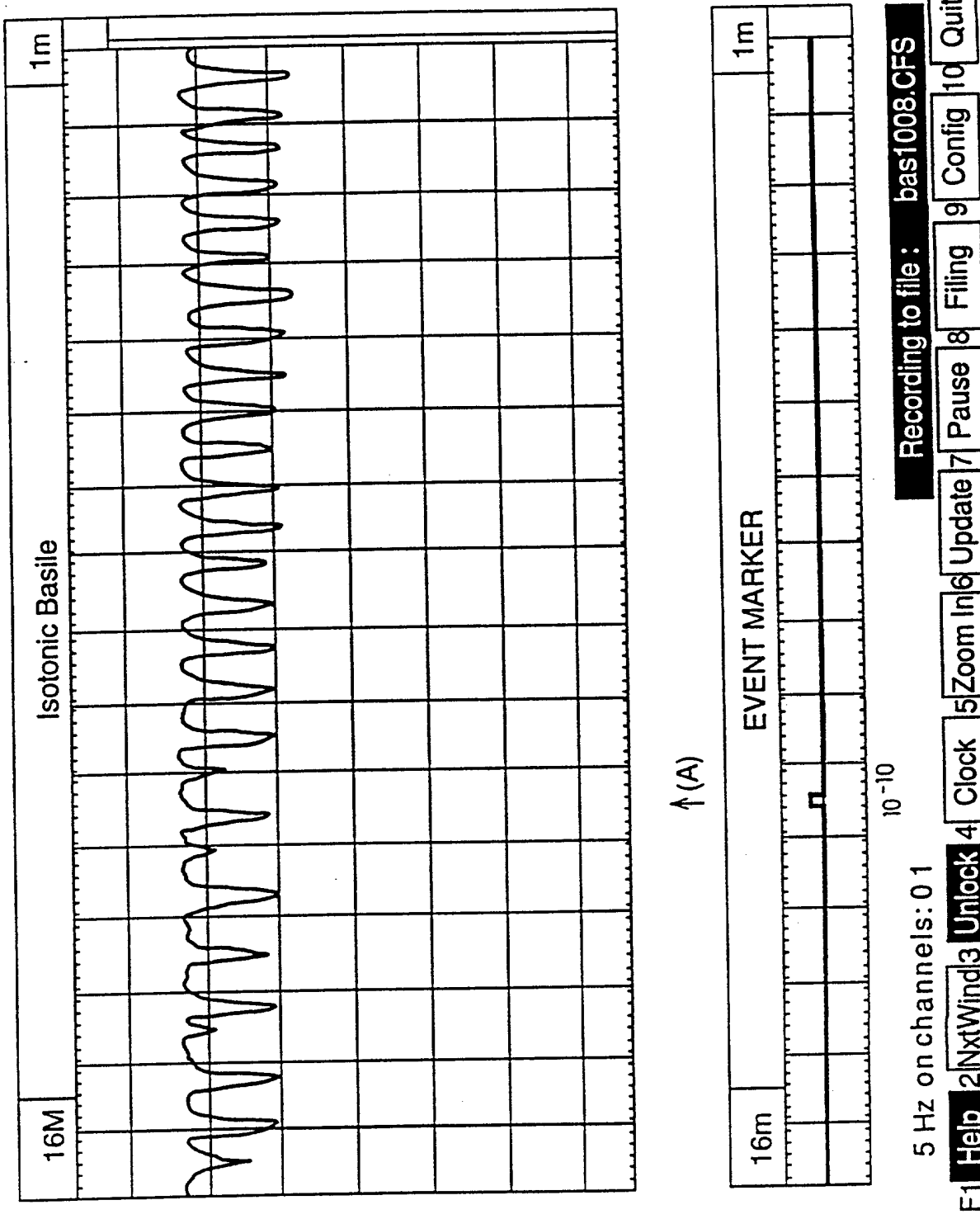

1m
Isotonic Basile
16M
↑(A)
1m
EVENT MARKER
16m
10^{-10}
Recording to file : bas1008.CFS
5 Hz on channels: 0 1
F1 Help 2 NxtWind 3 Unlock 4 Clock 5 Zoom In 6 Update 7 Pause 8 Filing 9 Config 10 Quit

FIG. 3(a)

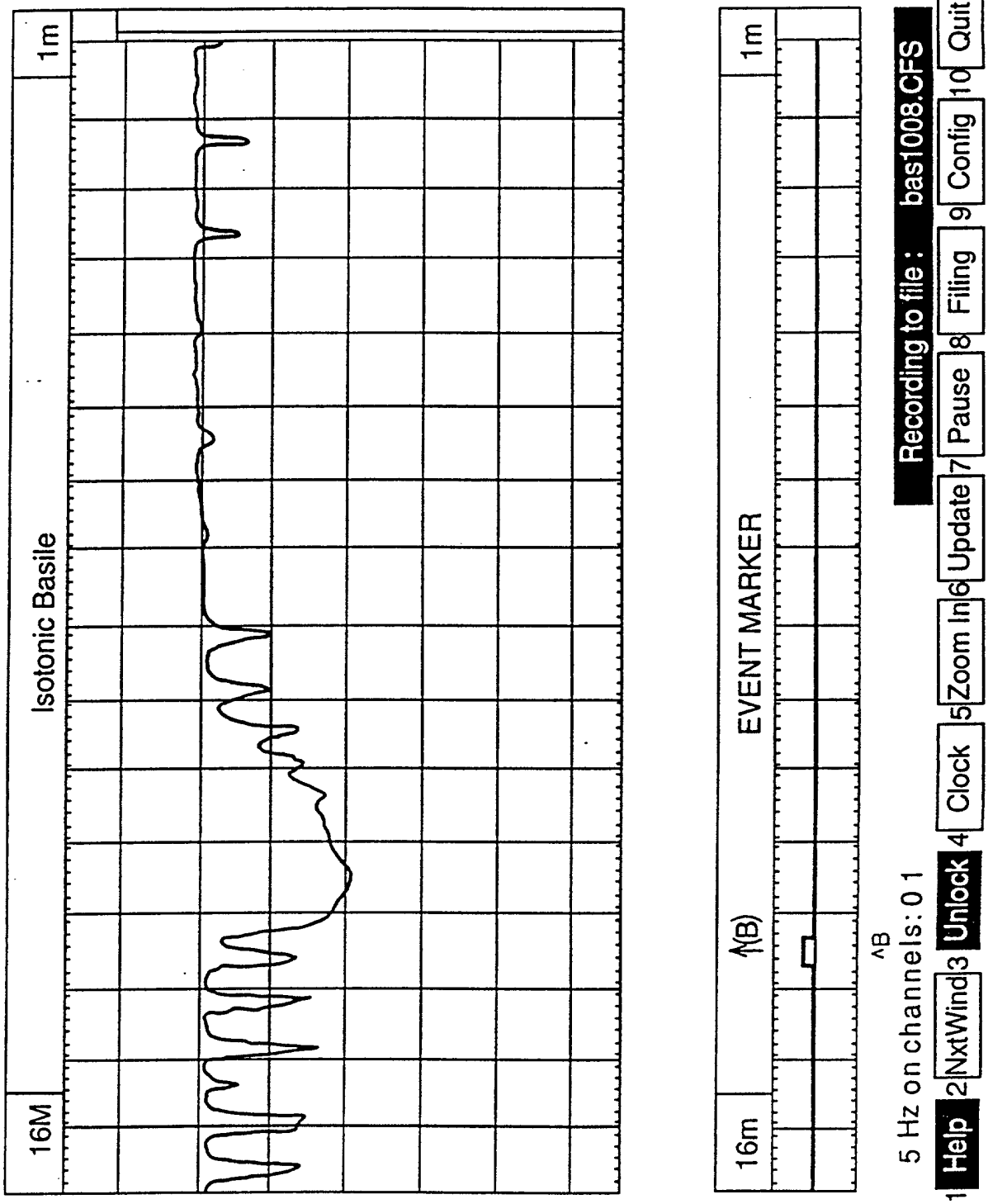

16M
Isotonic Basile
1m
16m
EVENT MARKER
1m
Recording to file : bas1008.CFS
5 Hz on channels: 0 1
F1 Help 2 NxtWind 3 Unlock 4 Clock 5 Zoom In 6 Update 7 Pause 8 Filing 9 Config 10 Quit

FIG. 3(b)

Docket No.: BKY 2 040

COMBINED DECLARATION FOR PATENT APPLICATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first, and joint inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

TYPE I ANGIOTENSIN II RECEPTOR SPECIFIC MONOCLONAL ANTIBODIES AND HYBRIDOMAS

the specification of which

____ is attached hereto.

____ was filed as United States application Serial No._____ _____ on _____ and was amended on_____

__X__ was filed as PCT international application No._____

PCT/GB94/02100 on 27 September 1994 and was amended under PCT Article 19 on _____

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, § 119 of any foreign application(s) for patent or inventor's certificate or of any PCT international application(s) designating at least one country other than the United States of America listed below and have also identified below any foreign application(s) for patent or inventor's certificate or any PCT international application(s) designating at least one country other than the United States of America filed by me on the same subject matter having a filing date before that of the application(s) of which priority is claimed:

Serial No.	Country	Filing Date
9319877.8	United Kingdom	27 September 1993

I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) or PCT international application(s) designating the United States of America that is/are listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, § 112. I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

NONE

As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith:

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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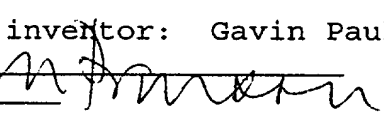
Inventor's signature 
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